APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2001, p. 5538–5543 Vol. 67, No. 12
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.12.5538–5543.2001

Ectomycorrhizal Fungal Associates of *Pinus contorta* in Soils Associated with a Hot Spring in Norris Geyser Basin, Yellowstone National Park, Wyoming

KEN CULLINGS[1]* AND SHILPA MAKHIJA[2]

NASA-Ames Research Center,[1] and San Francisco State University, c/o NASA-Ames Research Center,[2] Mountain View, California 94035

Received 29 May 2001/Accepted 27 September 2001

Molecular methods and comparisons of fruiting patterns (i.e., presence or absence of fungal fruiting bodies in different soil types) were used to determine ectomycorrhizal (EM) associates of *Pinus contorta* in soils associated with a thermal soil classified as ultra-acidic to extremely acidic (pH 2 to 4). EM were sampled by obtaining 36 soil cores from six paired plots (three cores each) of both thermal soils and forest soils directly adjacent to the thermal area. Fruiting bodies (mushrooms) were collected for molecular identification and to compare fruiting body (above-ground) diversity to below-ground diversity. Our results indicate (i) that there were significant decreases in both the level of EM infection (130 ± 22 EM root tips/core in forest soil; 68 ± 22 EM root tips/core in thermal soil) and EM fungal species richness (4.0 ± 0.5 species/core in forest soil; 1.2 ± 0.2 species/core in thermal soil) in soils associated with the thermal feature; (ii) that the EM mycota of thermal soils was comprised of a small set of dominant species and included very few rare species, while the EM mycota of forest soils contained a few dominant species and several rare EM fungal species; (iii) that *Dermocybe phoenecius* and a species of *Inocybe*, which was rare in forest soils, were the dominant EM fungal species in thermal soils; (iv) that other than the single *Inocybe* species, there was no overlap in the EM fungal communities of the forest and thermal soils; and (v) that the fungal species forming the majority of the above-ground fruiting structures in thermal soils (*Pisolithus tinctorius*, which is commonly used in remediation of acid soils) was not detected on a single EM root tip in either type of soil. Thus, *P. tinctorius* may have a different role in these thermal soils. Our results suggest that this species may not perform well in remediation of all acid soils and that factors such as pH, soil temperature, and soil chemistry may interact to influence EM fungal community structure. In addition, we identified at least one new species with potential for use in remediation of hot acidic soil.

Ectomycorrhizae (EM) are complex interactions between fungi and plant roots, are formed mainly by basidiomycete fungi (39, 47), and are the dominant nutrient-gathering organ in temperate ecosystems (45). These structures provide plants with nitrogen and phosphorus and protect plants from disease (18) and heavy metal contamination (34, 52). Different fungal species, and even isolates of the same species, can vary in their tolerance of harsh conditions (33, 50) and in the ability to help plants grow in extreme environments, such as acidic mine tailings (51) and coal spoils (24, 28). Because of these abilities, EM fungi are used to aid tree growth in programs designed to reclaim habitats altered by factors such as mining, nutrient deposition, and acid rain (11, 17). Thus, it has become increasingly important to determine in situ reactions of EM fungal communities to soil modifications that could inhibit plant growth.

Soil pH and temperature can affect EM fungal growth, fruiting body production and distribution, and plant growth and productivity (2, 25, 35, 36, 37, 43, 46). While EM typically form in acid soils, this process can be sensitive to pH values below 3.3 (16). pH and temperature growth optima (19, 29, 46, 51) for EM fungi can vary among species, even within a single genus (29, 30, 50). Similarly, increased soil temperature can adversely affect sclerotium formation in many EM fungal species, thus influencing inoculation potential (40), and can inhibit EM formation in both disturbed and undisturbed forest soils (43). Thus, pH tolerance and temperature tolerance are important criteria that should be considered when EM fungi are selected for soil reclamation and inoculation.

Our objectives were to determine conditions in thermal soils in a *Pinus contorta* forest in Yellowstone National Park, Wyoming, and to test the hypothesis that conditions in these soils significantly affect EM fungal infection, species richness, and EM fungal community structure. Because our results represent correlations between conditions and effects, additional studies involving manipulations may be required to fully test this hypothesis. To this end, soil cores were obtained from soil associated with an acidic thermal spring and from adjacent unmodified soils in a neighboring forest stand. These soils are acidic (pH 2 to 4), acid leached, and often less than 10 cm deep. Furthermore, the temperature in soils associated with Yellowstone's thermal features can increase with proximity to active hot springs to more than 60°C. Thus, these conditions are some of the harshest conditions for plant survival and EM fungal growth (42). Furthermore, additional soil cores targeted at individuals of the EM fungal species *Pisolithus tinctorius* were obtained to specifically determine the frequency of EM of this species. *P. tinctorius* is the EM fungal species most commonly

* Corresponding author. Mailing address: NASA-Ames Research Center, Mountain View, CA 94035. Phone: (650) 604-2773. Fax: (650) 604-1088. E-mail: kcullings@mail.arc.nasa.gov.

TABLE 1. Organic matter and nutrient levels in forest and thermal soils

Soil	Concn (mean ± SE) of:								
	Organic matter (%)	NH_4 (ppm)	NO_3 (ppm)	Ca (meg/liter)	Mg (meg/liter)	P (ppm)	K (ppm)	Total N (%)	Al (ppm)
Forest	6.72 ± 0.03	14 ± 0.7	0.70 ± 0.05	0.62 ± 0.70	0.24 ± 0.03	47 ± 6.2	180 ± 4.2	0.24 ± 0.02	8,900 ± 370
Thermal	2.9 ± 0.6[a]	19 ± 1.3[b]	0.68 ± 0.13[c]	3.7 ± 1.8[b]	0.90 ± 0.30[d]	29 ± 8.8[c]	100 ± 13[b]	0.13 ± 0.05[d]	4,700 ± 610[d]

[a] Significantly different from the forest soil value ($P < 0.001$), as determined by the Mann-Whitney U test.
[b] Significantly different from the forest soil value ($P < 0.05$), as determined by the Mann-Whitney U test.
[c] Not significantly different from the forest soil value, as determined by Mann-Whitney U test.
[d] Significantly different from the forest soil value ($P < 0.01$), as determined by the Mann-Whitney U test.

used in remediation of acidic soils (56), and we wanted to determine whether this species was the dominant EM former in this eco-system.

MATERIALS AND METHODS

The study site was located in the Norris Geyser Basin of Yellowstone National Park, Wyoming. Soil cores were obtained both from soils associated with a hot spring and from nonthermal soils in an adjacent forest. The soil temperatures were 37 ± 2.6°C in all coring plots in the thermal soils and 17 ± 1.5°C in the forest soils. Temperature measurements were taken on 2 days in July, at noon on both days, in the shade and under the litter of at least three trees per plot to minimize the effects of solar heating. The pH values in the plots of thermal and forest soils were 3.3 ± 0.1 and 4.02 ± 0.01, respectively. There were no understory plants present at either location.

For each soil type, cores that were 18.8 cm in diameter and 10 cm deep were taken; three cores were obtained from each of six replicate paired (thermal-nonthermal) plots situated along two parallel 100-m transects that were separated by less than 30 m. A total of 36 soil cores were taken. The coring depth was based on the depth of roots in the soils and was chosen to provide equal volumes of soil for statistical analyses. The cores were sifted to remove the soil, and mycorrhizae were separated on the basis of color (1) and then stored dry at −20°C within 10 days of collection. The EM tips of each morphotype in each core were pooled for quantification (14, 15, 27) and then freeze-dried for long-term storage. No samples were stored alive for deposition in international culture collections, although all morphotypes from all cores were archived for future analyses.

Fungi that formed individual EM were identified by PCR-based methods. To ensure that genetic variability within morphotypes was not missed because of undersampling, individual mycorrhizae were selected for DNA analysis by using the following scheme: one tip per core was analyzed if a morphotype was represented by one to three individual mycorrhizae per core, three tips per core were analyzed if a morphotype was represented by three to five mycorrhizae per core, and five tips per core were analyzed if a morphotype was represented by more than five individual mycorrhizae per core. DNA was amplified from root tips and from fruiting bodies by using primers and ITS1F and ITS4B, which were specific for the internal transcribed spacer region of the nuclear rRNA repeat unit of basidiomycete fungi (22). To identify ascomycete EM fungi, the universal ITS1F-ITS4 fungal primer set was used (22). We used the following parameters for PCR (12): initial denaturation at 95°C for 1 min 35 s, followed by 13 cycles of denaturation for 35 s at 94°C, primer annealing for 55 s at 55°C, and polymerization for 45 s at 72°C, nine additional cycles in which the polymerization time was extended to 2 min, nine cycles consisting of 3 min of extension, and a final 10-min polymerization step at 72°C.

Amplified DNA was digested with restriction enzymes *Alu*I and *Hin*fI, and the band patterns obtained from EM were compared to those obtained from fruiting bodies. Restriction fragment length polymorphism (RFLP) patterns that were identical for EM and fruiting bodies were considered a match; the utility and accuracy of this method have been demonstrated previously (23), and this method was used by us previously in Yellowstone National Park studies (8, 14, 15). When fungal species comprising more than 4% of the EM fungal community could not be identified by using fruiting bodies, the fungi were identified as members of family level monophyletic groups by amplifying a portion of the mitochondrial large rRNA subunit with PCR primers ML5 and ML6 and subsequently comparing the DNA sequences amplified from EM root tips to sequences in a previously published database (7). It is often the case in EM systems that fungal species that are common below ground do not form fruiting bodies; hence, identification beyond a family level monophyletic group is often impossible (23).

Fruiting bodies (mushrooms) of EM species were collected throughout the growing season (June to September) in a 10- by 10-m area around each collection plot in both the thermal and nonthermal soils. Very few fungal species fruited in the collection plots in thermal soils. Thus, fruiting bodies that we collected for other studies in nearby stands (8, 14, 15) were used to identify EM fungi in the thermal soils. Only fruiting bodies collected in collection plots were used to compare fruiting patterns in the two soil types.

Soil chemistry (pH and organic matter, total nitrogen, ammonium, nitrate, phosphorus, potassium, calcium, and aluminum contents) of both the thermal and forest soils was analyzed by the DANR Analytical Laboratory, University of California at Davis, Davis, Calif. As the instructions recommended, soils were collected, oven dried at 55 to 60°C, sieved through a 2-mm mesh, and approximately 300-g portions of soil were collected for analysis. The pH was determined with a pH meter after aqueous extraction (55). The organic matter content was determined by potassium dichromate reduction of organic carbon and subsequent spectrophotometric measurement (modified Walkley-Black method) (41). The soluble calcium and magnesium contents in a saturated paste extract were determined by inductively coupled plasma atomic emission spectrometry (33). The aluminum content was determined by acid dissolution (49). The P Olsen content was determined by alkaline extraction with 0.5 N $NaHCO_3$. The P Bray content was determined by extraction for acid soils (pH less than 7.0) by using a dilute acid-fluoride extractant (26). The soluble potassium content in the saturated paste extract was determined by emission spectrometry (33). The total nitrogen content was determined by combustion (44, 45), and the nitrate and ammonium contents were determined by extraction with potassium chloride and subsequent measurement with a diffusion-conductivity analyzer (31).

Differences in species richness (number of EM fungal species per core) and level of EM infection (number of individual EM per core) in the two soil types were examined by a Mann-Whitney U test. Differences in overall EM fungal community composition, both above ground based on fruiting body comparisons and below ground based on internal transcribed spacer-RFLP analysis, were examined by using a contingency table and a chi-square test; in the case of below-ground EM fungal diversity, this test was done by using both cores in which each fungal species was detected and the number of EM in each core. Differences in soil chemistry were examined by using Student's *t* test.

A preliminary molecular analysis (six cores) failed to detect *P. tinctorius* EM in the thermal soils. Therefore, in addition to the 18 cores taken from the thermal soils for assessment of the EM community, six additional cores were taken directly under fruiting bodies of this species in order to determine the frequency of occurrence of *P. tinctorius* EM in the vicinity of fruiting bodies of this species.

RESULTS

The analysis of soil chemistry (Table 1) indicated that the thermal soils were significantly more acidic, contained less organic matter, and had lower levels of phosphorus, potassium, aluminum, and total nitrogen. The ammonium and magnesium contents were significantly higher in the thermal soils, as was the calcium content. The nitrate contents of the soils did not differ significantly.

Statistical analyses of individual EM root tips indicated that both species richness (4.0 ± 0.5 species/core in nonthermal soils, 1.2 ± 0.2 species/core in thermal soils) and the level of EM infection (130 ± 22 EM root tips/core in nonthermal soils, 68 ± 22 EM root tips/core in thermal soils) were lower in the thermal soils ($P < 0.005$, df = 34).

TABLE 2. EM occurrence on root tips

Fungal species	No. of plots[a]	% of community	*Alu*I RFLP pattern (bp)	*Hin*fI RFLP pattern (bp)
Thermal soil EM[b]				
Dermocybe phoeniceus	5	45	404, 190, 150	320, 320[c]
Inocybe H22[d]	2	27	320, 250, 110	600
NMT1Basidio 1[e]	4	21	410, (250), 160, 120[f]	242, 200, 130, 110
Lactarius rufus	2	4.4	500, 170, 125, 80	410, 250, 124, 80
NMT1Basidio 2	1	0.9	440, 330	260, 215, 125, 70
Ascomycete T13	1	0.8	404, 130	330, 260
Sarcodon imbricatus	1	0.5	330, 240, 190, 70	330, 170, 140, 100
Forest soil EM				
Cantharellaceae H1	2	20	380, 290, 80	340, 330, 130
Rhizopogon JJ	5	19	400, 220	380, 160
Agaricaceae H21	2	11	480, 300	380, 64
Cortinarius H2	2	10	500, 100	380, 340, 124
H25[e]	1	9.2	320, 220	400, 300, 124
Cenococcum A2	6	9.1	400, 190	340, 300, 160, 124, 110
Tricholomataceae H20	2	3.9	400, 90	404, 90
H11	1	3.4	260, 220, 100	300, 300
H4	1	2.6	410, 410	320, 320, 110
H23	1	2.5	320, 220	360, 242
H28	1	2.1	350, 330	500, 300
H27	1	2.0	380, 320	700
Cortinarius H12	1	1.6	450, 320	500
A10[g]	1	1.0	360, 340	650,
Cortinariaceae H32	1	1.0	380, 220	520, 300
NMF2A1	1	0.94	404, 215	300, 175
NMF2A2	1	0.93	505, 360	600, 160, 100
A5	1	0.84	520	340, 320
H17	1	0.68	320, 180, 150, 100	600, 150
H33	1	0.68	360, 340, 100	300, 260, 190
NMF2B1	1	0.52	310, 150, 70	260, 200, 125
Inocybe H22	1	0.52	320, 250, 110	600
H18	1	0.42	340, 200, 130, 50	400, 280, 80
Suillus tomentosus H24	1	0.42	220, 190, 120, 67	400, 300
A6	1	0.31	350, 340, 70	650, 150
A13	1	0.27	400, 320	380, 160
H8	1	0.10	320, 180, 140	400, 300

[a] A total of six plots were used for each soil type.
[b] Eighteen cores were sampled for each soil type.
[c] The RFLP band sizes often do not add up to the size of the amplified product (750 to 900 bp) because some patterns contained several small bands whose sizes could not be accurately determined.
[d] Designations such as H1 and NMT1 are used to cross-reference basidiomycete fungi to samples in our database.
[e] EM fungus that accounted for more than 5% of the EM community with no fruiting body match and for which ML5-ML6 amplification failed.
[f] Parentheses indicate a faint, but informative, RFLP band.
[g] The prefix A indicates an ascomycete fungus, whose RFLP pattern was based on ITS1F-ITS4 amplification.

Molecular analysis indicated that the EM fungal communities of the forest soils contained a diverse array of fungi, including basidiomycetes belonging to both the Agaricales and the Boletales and the ascomycete *Cenococcum* (Table 2); contingency table and chi-square analysis indicated that there was a significant difference between the EM fungal communities of the thermal soils and the EM fungal communities of the unmodified forest soils ($P < 0.001$). Furthermore, while the forest soils contained a few dominant EM fungal species that were accompanied by several rare species, the thermal soils contained three dominant fungal species that accounted for 93% of the community and only four rare taxa (Table 2).

The ascomycete *Cenococcum* was common in the forest soils. The remaining dominant species were members of several basidiomycete families (members of the orders Agaricales and Boletales), including the Suilloid group, the Cantharellaceae, and the Cortinariaceae. In contrast, the EM fungal community of the thermal soils was dominated by fungi in the Cortinariaceae and by a basidiomycete species that failed to form fruiting bodies in either type of soil and could not be identified by sequence as a member of any family level monophyletic group due to the presence of a large intron. The dominant EM fungal species of the thermal soils were *Dermocybe phoeniceus* and a species of *Inocybe* designated *Inocybe* H22. Together, these two species comprised 71% of the EM fungal community. Only one species, *Inocybe* H22, was present in both the thermal soils and the forest soils. This species was present in the forest soils but at a low level, comprising less than 1% of the EM fungal community. Unfortunately, *Inocybe* H22 was cryptic and could not be definitively identified to the species level, but it was a member of subsection Marginatae (defined by nodulose or angular spores), section *Inocybe* (defined by the presence of caulocystidia along the entire length of the stipe).

The levels of fruiting body diversity in the sampling plots for the two soil types were significantly different ($P < 0.001$). In forest soils, genera belonging to 14 families were represented; these families included the Amanitaceae (*Amanita*), the Bole-

taceae (*Boletus*, *Leccinum*, *Rhizopogon*, and *Suillus*), the Cantharellaceae (*Cantharellus*), the Clavariaceae (*Ramaria*), the Cortinariaceae (*Cortinarius*, *Dermocybe*, *Hebeloma*, and *Inocybe*), the Entolomataceae (*Entoloma*), the Gomphidiaceae (*Chroogomphus* and *Gomphidius*), the Hygrophoraceae (*Hygrophorus*), the Lycoperdaceae (*Lycoperdon*), the Polyporaceae (*Albatrellis*), the Russulaceae (*Lactarius*, including *Lactarius rufus*, and *Russula*), the Strobilomycetaceae (*Gautiera*), the Thelephoraceae (*Thelephora* and *Sarcodon*), and the Tricholomataceae (*Tricholoma*, *Tricholomopsis*, *Tyromyces*, and *Xeromphalina*). The fruiting body diversity in the thermal soils was greatly reduced, although fruiting bodies of most species detected below ground were collected. The fungi fruiting in the thermal soils included members of the Cortinariaceae (*Inocybe* H22), the Pisolithaceae (*P. tinctorius*), the Russulaceae (*L. rufus*), and the Thelephoraceae (*Sarcodon imbricatus*).

Surprisingly, even though *P. tinctorius* formed several fruiting bodies in the thermal soils, no EM of *P. tinctorius* were detected in any of the 36 cores taken from paired plots in either soil type. Analyses of six additional cores taken directly under *P. tinctorius* fruiting bodies also failed to locate a single root tip that formed EM with this fungal species.

DISCUSSION

Not only were the thermal soils hotter than the forest soils, but they were also more acidic, contained less organic matter, and had different levels of important soil ions, such as ammonium, calcium, and aluminum. This combination of conditions was accompanied by less below-ground species richness, lower levels of EM infection, and the absence of the large number of rare species that were detected in the undisturbed forest soils. In addition, the number of higher-order taxa (family level and higher) was smaller in the thermal soils, and the composition of the EM fungal community was significantly different. The thermal soils were dominated by two species belonging to the Cortinariaceae (*D. phoenecius* and *Inocybe* H22), which together accounted for more than 70% of the total EM fungal community. *D. phoenecius* was the dominant species, accounting for 45% of the total thermal soil EM fungal community, but this species was absent from the forest soils. *Inocybe* H22 (26% of the total thermal soil community) was very rare in the forest soils (<1% of the total community); no other EM fungal species were detected in both types of soil. Both of these fungi are common, have broad host ranges, and have wide geographic distributions (32, 43). Their overall flexibility probably allowed these species to thrive in the thermal soils and makes them possible alternatives for reclamation studies involving *P. contorta*.

While the pH was lower in the thermal soils, the effects on the EM fungal community were probably not due to pH differences alone as EM fungal species are common in acid soils and many EM fungal species grow well at a pH of 3.3 or less (3, 38, 53). Indeed, some conditions resulting from the increased acidity in thermal soils (e.g., decreased aluminum content and increased calcium content) can enhance growth and nutrient uptake by some EM fungi (6, 30). We think that the detrimental effects on the EM fungal community probably were due to increased soil temperature and/or reduced organic matter content. The growth of most EM fungal species that have been tested in culture is reduced at temperatures below the temperature measured in the thermal soils examined (37°C) (36, 50). Thus, despite the potentially positive effects of the decreased aluminum content and increased calcium content in the thermal soils, the level of EM infection and species richness were both reduced in the presence of higher soil temperatures. For example, the ascomycete fungus *Cenococcum* tolerates high acidity (16, 35), is present at relatively high concentrations in forest soils, can exhibit enhanced growth in response to acid rain conditions (38), is sensitive to increased temperatures, exhibits optimum growth at 16 to 27°C in culture, and does not grow at temperatures above 38°C (10). In contrast, although the levels of *Sarcodon* species are low (only 1% of the community), these organisms tolerate increased soil temperatures and can help host trees grow in harsh environments, such as coal spoils (28).

Similarly, although the effects are likely to be more subtle than the growth limitation observed at temperatures at or above those measured in the soils studied, reduced levels of organic matter (and hence lower levels of organic nitrogen) can also favor some species while inhibiting others. The level of organic matter was lower in the thermal soils, and species of the genera *Cenococcum* and *Suillus* (both detected only in the forest soils with higher levels of organic matter) are more productive when they are grown on organic sources of nitrogen. Other fungi (e.g., *L. rufus*, which was detected only in the thermal soils) are more productive when they are grown on inorganic nitrogen sources (e.g., ammonium, which was present at significantly higher levels in the thermal soils than in the forest soils) (53). *L. rufus* was present at a relatively low frequency in the thermal soils but was absent from the forest soils, can associate with both *Pinus* and *Picea* species, and grows well in boggy, acidic habitats (3). Thus, the low level of *L. rufus* in thermal soils may seem confusing. However, although *L. rufus* prefers inorganic sources of nitrogen, growth of this species can be inhibited at low pH by increased ammonium levels, such as those detected in the thermal soils (29). We hypothesize that the interaction between low pH and increased ammonium content in the thermal soils prevented *L. rufus* from becoming a dominant organism, despite its preference for acidic soils. Although there have been no studies of *D. phoeniceus* and *Inocybe* H22 in relation to harsh soils, these organisms may respond to combined organic matter-pH-soil temperature relationships in a similar manner. Targeted studies of these species in relation to these factors are needed to determine if this is the case, and data indicate that these fungi may be candidates for use in reclamation of some harsh acidic soils.

The fruiting bodies collected indicated that above-ground (apparent) EM fungal diversity was reduced in the thermal soils. *P. tinctorius* formed the majority of the fruiting bodies in the thermal feature. This result was not surprising; *P. tinctorius* grows well in culture at temperatures up to 40°C, tolerates high acidity (10, 32, 36, 37, 50), and is the fungal species most commonly used to help remediate severely altered soils (54). Yet we did not detect *P. tinctorius* on a single EM root tip from either soil type. Targeted assays of EM obtained directly under *P. tinctorius* fruiting bodies also failed to detect EM of this fungal species. Therefore, it is unlikely that we missed EM of *P. tinctorius* simply due to sampling error. Why this species was

not detected is not clear, although culture experiments have demonstrated that *P. tinctorius* can be either mycorrhizal or not mycorrhizal depending on the sugar conditions in the medium or possibly on the stage of development of the host plant (21). For example, the quality and quantity of exogenous sugars can strongly influence EM development; hence, the low organic matter content of our thermal soils could have inhibited growth of this species. In addition, EM fungi demand significant portions of the sugars fixed by their host plants via photosynthesis (4, 20), and the *P. contorta* individuals growing in the harsh conditions studied may have been under sufficient stress that they could not provide *P. tinctorius* with sufficient carbon. Regardless of the mechanism behind the pattern observed here, *P. tinctorius* must obtain carbon to form fruiting bodies, although the source of this carbon is not known.

A similar pattern of copious fruiting and rare EM occurrence has been observed in another EM fungal genus, *Suillus* (23), and two hypotheses to explain this phenomenon have been advanced. The first hypothesis is that there is a very efficient mechanism to transfer carbon via coevolved recognition systems between the plant and fungal partners (23). Up to 50% of the total carbon fixed by photosynthesis is passed to the fungal associates (20), and it is possible that highly specific systems could enhance this transfer by providing a more efficient avenue of nutrient transfer (13), enabling fungal growth via very few, difficult-to-locate EM connections. However, *P. tinctorius* is not considered to be highly host specialized (38), which makes this explanation less likely. An alternate hypothesis is that EM fungi obtain carbon through saprophytic growth (23). Many EM fungi possess the enzymes and transport mechanisms to break down forest litter and acquire metabolizable nitrogen and carbon (9). Despite the low organic matter content of thermal soils, the lack of association of *P. tinctorius* with roots of *P. contorta* suggests that *P. tinctorius* may indeed be capable of enzymatically breaking down organic substrates. Targeted studies of the enzymatic capabilities of *P. tinctorius* in this system are required to test this hypothesis. Furthermore, further study is needed to determine the role of *P. tinctorius* in this ecosystem. Our results indicate that this species may not be suitable for EM-related rehabilitation projects in all low-pH–high-temperature soils.

In summary, our results indicate that chemical changes in acidic thermal areas can significantly alter EM fungal community structure. In thermal areas of Yellowstone National Park, the conditions can vary greatly over short distances (48), and as a result, pH-neutral regions can occur close to acidic hot springs. These small adjacent regions are subject to the same input of fungal inoculum from adjacent forest stands. Thus, Yellowstone's thermal features could act as natural field laboratories for studying the physiological potential of EM fungi by providing a range of chemical conditions in which the adaptive abilities of the fungi could be assessed at spatial scales which ensure that the variability in the EM fungal community is due less to spatial variation than to soil properties.

ACKNOWLEDGMENTS

This work was supported by a NASA Director's Discretionary Fund grant to Ken Cullings.

We thank J. R. Blair for mushroom identification and Bob Lindstrom of the Yellowstone Center for Resources and Ann Rodman of the Yellowstone Soil Survey for field support.

REFERENCES

1. **Agerer, R.** 1987. Colour atlas of ectomycorrhizae. Einhorn-Verlag, Eduard Dietenberger, Schwabisch-Gmünd.
2. **Agerer, R., A. F. S. Taylor, and R. Treu.** 1998. Effects of acid irrigation and liming on the production of fruit bodies by ectomycorrhizal fungi. Plant Soil **199:**83–89.
3. **Arora, D.** 1986 Mushrooms demystified. Ten Speed Press, Berkeley, Calif.
4. **Bradley, R., A. J. Burt, and D. J. Read.** 1981. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. Nature **292:**335–337.
5. **Bradley, R., A. J. Burt, and D. J. Read.** 1982. The biology of mycorrhizae in the Ericaceae. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytol. **91:**197–209.
6. **Browning, M. H. R., and T. C. Hutchinson.** 1990. The effects of aluminum and calcium on the growth and nutrition of selected ectomycorrhizal fungi of jack pine. Can. J. Bot. **69:**1691–1699.
7. **Bruns, T. D., T. M. Szaro, M. Gardes, K. W. Cullings, J. J. Pan, D. L. Taylor, T. R. Horton, A. Kretzer, M. Garbelotto, and Y. U. Li.** 1998 A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol. Ecol. **7:**257–272.
8. **Byrd, K. B., V. T. Parker, D. R. Vogler, and K. W. Cullings.** 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can. J. Bot. **78:**149–156.
9. **Chalot, M., and A. Brun.** 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiol. Rev. **22:**21–44.
10. **Cline, M. L., R. C. France, and C. P. Reid.** 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. Can. J. Bot. **65:**869–875.
11. **Cordell, C. E.** 1997. Mycorrhizal fungi: beneficial tools for mineland reclamation and Christmas trees. U.S. For. Serv. Gen. Tech. Rep. PNW **389:**91–92.
12. **Cullings, K. W.** 1996. Single phylogenetic origin of ericoid mycorrhizae within the Ericaceae. Can. J. Bot. **74:**1896–1909.
13. **Cullings, K. W., T. M. Szaro, and T. D. Bruns.** 1996. Evolution of extreme specialization within a lineage of ectomycorrhizal epiparasites. Nature **379:**63–66.
14. **Cullings, K. W., V. T. Parker, S. K. Finley, and D. R. Vogler.** 2000. Spatial-temporal dynamics and specificity of ectomycorrhizal interactions in Yellowstone forests. Appl. Environ. Microbiol. **66:**4988–4991.
15. **Cullings, K. W., D. R. Vogler, V. T. Parker, and S. Makhija.** 2001. Effects of artificial defoliation on the ectomycorrhizal community of a mixed *Pinus contorta*/*Picea engelmannii* stand in Yellowstone Park. Oecologia **127:**533–539.
16. **Danielson, R. M., and S. Visser.** 1989. Effects of forest soil acidification on ectomycorrhizal and vesicular-arbuscular mycorrhizal development. New Phytol. **112:**41–47.
17. **Dodd, J. C., and B. D. Thomson.** 1994. The screening and selection of inoculant arbuscular-mycorrhizal and ectomycorrhizal fungi. Plant Soil **159:**149–158.
18. **Duchesne, L. C., B. E. Ellis, and R. L. Peterson.** 1989. Disease suppression by the ectomycorrhizal fungus *Paxillus involutus*: contribution of oxalic acid. Can. J. Bot. **67:**2726–2730.
19. **Erland, S., and R. Finlay.** 1992. Effects of temperature and incubation time on the ability of three ectomycorrhizal fungi to colonize *Pinus sylvestris* roots. Mycol. Res. **96:**270–272.
20. **Finlay, R., and B. Soderstrom.** 1992. Mycorrhiza and carbon flow to the soil, p. 134–162. *In* M. Allen (ed.), Mycorrhizal functioning, an integrative plant-fungal process. Chapman & Hall, New York, N.Y.
21. **Garbaye, B. A. M., and J. Dexheimer.** 1994. The influence of culture conditions on mycorrhizae formation between the ectomycorrhizal fungus *Pisolithus* sp. and *Afzelia africana* Sm. seedlings. Mycorrhiza **4:**121–129.
22. **Gardes, M., and T. D. Bruns.** 1993. ITS primers with enhanced specificity for higher fungi and basidiomycetes: application to identification of mycorrhizae and rusts. Mol. Ecol. **2:**113–118.
23. **Gardes, M., and T. D. Bruns.** 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Can. J. Bot. **74:**1572–1583.
24. **Gardner, J. G., and N. Malajczuk.** 1988. Recolonization of rehabilitated bauxite mine sites in western Australia by mycorrhizal fungi. For. Ecol. Manage. **24:**27–42.
25. **Honrubia, M., and G. Diaz.** 1996. Effect of simulated acid rain on mycorrhizae of Aleppo pine (*Pinus halepensis* Miller) in calcareous soil. Ann. Sci. For. **53:**947–954.
26. **Horneck, D. A., Hart, J. M., K. Topper, K., and B. Koespell.** 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Agric. Exp. St. Soil Methods **89:**4.
27. **Horton, T. R., and T. D. Bruns.** 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytol. **139:**331–339.

28. **Ingleby K., F. T. Last, and P. A. Mason.** 1985. Vertical distribution and temperature relations of sheathing mycorrhizas of *Betula* spp. growing on coil spoil. For. Ecol. Manage. **12:**279–285.
29. **Jongbloed, R. H., and G. W. F. Borst-Pauwels.** 1990. Effects of ammonium and pH on growth of some ectomycorrhizal fungi *in vitro*. Acta Bot. Neerl. **39:**349–358.
30. **Jongbloed, R. H. and G. W. F. H. Borst-Pauwels.** 1992. Effects of aluminum and pH on growth and potassium uptake by three ectomycorrhizal fungi in liquid culture. Plant Soil **140:**157–165.
31. **Keeney, D. R., and D. W. Nelson.** 1982. Nitrogen—inorganic forms, p. 643–698. *In* A. L. Page (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. Monograph number 9, 2nd ed. ASA, Madison, Wis.
32. **Kowalski, S., E. Obloza, and W. Wojewoda.** 1996. Susceptibility of ectomycorrhizal and ectendomycorrhizal fungi to pH of the environment. Acta Mycol. **31:**127–136.
33. **Lanyon, L. E., and W. R. Heald.** 1982. Magnesium, calcium, strontium, and barium, p. 247–262. *In* A. L. Page (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. Monograph number 9, 2nd ed. ASA, Madison, Wis.
34. **Leyval, C., K. Turnau, and K. Haselwandter.** 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza **7:**139–153.
35. **Marx, D. H., and B. Zak.** 1965. Effect of pH on mycorrhizal formation of slash pine in aseptic culture. For. Sci. **11:**65–75.
36. **Marx, D. H., C. W. Bryan, and C. B. Davey.** 1970. Influence of temperature on aseptic synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on loblolly pine. For. Sci. **16:**424–431.
37. **Marx, D. H., and W. C. Bryan.** 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings in loblolly pine at high temperature. For. Sci. **17:**37–41.
38. **Meier, S., W. P. Robarge, R. I. Bruck, and L. F. Grand.** 1989. Effects of simulated rain acidity on ectomycorrhizae of red spruce seedlings potted in natural soil. Environ. Pollut. **59:**315–324.
39. **Molina, R., J. Massicotte, and J. M. Trappe.** 1992. Specificity phenomena in mycorrhizal symbioses: community ecological consequences and practical applications, p. 357–420. *In* M. Allen (ed.), Mycorrhizal functioning, an integrative plant-fungl process. Chapman and Hall, New York, N.Y.
40. **Moore, A. E. P., and R. L. Peterson.** 1992. Effect of temperature on sclerotium induction in *Paxillus involutus*. Can. J. Microbiol. **38:**1197–1201.
41. **Nelson, D. W., and L. E. Sommers.** 1982. Total carbon, organic carbon, and organic matter, p. 539–579. *In* A. L. Page (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. Monograph number 9, 2nd ed. ASA, Madison, Wis.
42. **Olsen, S. R., C. V. Cole, F. S. Watanabe and L. A. Dean.** 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. Agric. Circ. **939:**1–19.
43. **Parke, J. L., R. G. Linderman, and J. M. Trappe.** 1983. Effect of root zone temperature on ectomycorrhiza and vesicular-arbuscular mycorrhiza formation in disturbed and undisturbed forest soils of southwest Oregon. Can. J. For. Res. **13:**657–665.
44. **Pella, E.** 1990. Elemental organic analysis, part 1: historical developments. Am. Lab. **22:**116.
45. **Pella, E.** 1990. Elemental organic analysis, part 2: state of the art. Am. Lab. **22:**28.
46. **Pokojska, A., M. Kampert, H. Rozycki, and E. Strelczyk.** 1996. Effects of vitamins, temperature and pH on the biomass-production by ectomycorrhizal fungi. Acta Mycol. **31:**55–65.
47. **Read, D. J.** 1991. Mycorrhizas in ecosystems. Experientia **47:**376–391.
48. **Rodman, A., H. F. Shovic, and D. Thoma.** 1996. Soils of Yellowstone National Park. Publication YCR-NRSR-96–2. Yellowstone Center for Resources, Yellowstone National Park, Wyo.
49. **Sah, R. N., and R. O. Miller.** 1992 Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Anal. Chem. **64:**230–233.
50. **Samson, J., and J. A. Fortin.** 1986. Ectomycorrhizal fungi of *Larix laricina* and the interspecific and intraspecific variation in response to temperature. Can. J. Bot. **64:**3020–3028.
51. **Senior, E., J. E. Smith, I. A. Watson-Craik, and J. E. Tosh.** 1993. Ectomycorrhizae and landfill site reclamations: fungal selection criteria. Lett. Appl. Microbiol. **16:**142–146.
52. **Shaw, G., A. J. M. Leake, and D. J. Read.** 1990. The biology of mycorrhizae in the Ericaceae. XVII. The role of mycorrhizal infection in the regulation of iron uptake by ericaceous plants. New Phytol. **115:**251–258.
53. **Smith, S. E., and D. J. Read.** 1997. Mycorrhizal symbiosis. Academic Press, London, United Kingdom.
54. **Tam, C. F.** 1995. Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza **5:**181–187.
55. **U. S. Salinity Laboratory Staff.** 1954. Diagnosis and improvement of saline and alkali soils. U.S. Department of Agriculture Handbook. U.S. Department of Agriculture, Washington, D.C.
56. **Walker, R. F.** 1990. Formation of *Pisolithus tinctorius* ectomycorrhizae on California white fir in an eastern Sierra Nevada mine soil. Great Basin Nat. **50:**85–87.